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Pros and cons of phage therapy

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Many publications list advantages and disadvantages associated with phage therapy, which is the use of bacterial viruses to combat populations of nuisance or pathogenic bacteria. The goal of this commentary is to discuss many of those issues in a single location. In terms of "Pros," for example, phages can be bactericidal, can increase in number over the course of treatment, tend to only minimally disrupt normal flora, are equally effective against antibiotic-sensitive and antibiotic-resistant bacteria, often are easily discovered, seem to be capable of disrupting bacterial biofilms, and can have low inherent toxicities. In addition to these assets, we consider aspects of phage therapy that can contribute to its safety, economics, or convenience, but in ways that are perhaps less essential to the phage potential to combat bacteria. For example, autonomous phage transfer between animals during veterinary application could provide convenience or economic advantages by decreasing the need for repeated phage application, but is not necessarily crucial to therapeutic success. We also consider possible disadvantages to phage use as antibacterial agents. These "Cons," however, tend to be relatively minor.

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Introduction

Introduced in the early 1900s,[1] phage therapy is the application of bacteria-specific viruses (phages) to combat uncontrolled and undesired bacteria such as those associated with infectious disease.[2] In reviews of phage therapy[3] authors commonly list advantages of employing phages as antibacterials (for example, see ref. 4). These lists can be used as talking points of why, in this age of epidemic antibiotic resistance, phage therapy should not be overlooked. As lists vary from author to author, it is useful to condense them into a coherent whole. Here we highlight the strengths and weaknesses of individual assertions. We also consider possible limitations to phage use as antibacterials. A more comprehensive review of phage therapy is presented in this same issue while this commentary focuses expressly on the pros and cons of phage use as antibacterials.

Major Advantages of Phage Therapy

Advantages of phage therapy over the use of chemical antibiotics can be framed in terms of phage properties. In this section we consider those properties that, in our opinion, can contribute substantially to phage therapy utility.

Bactericidal agents. Bacteria that have been successfully infected by obligately lytic phages are unable to regain their viability. By contrast, certain antibiotics are bacteriostatic, such as tetracycline, and as a consequence may more readily permit bacterial evolution towards resistance.[5,6]

Auto "dosing". Phages during the bacterial-killing process are capable of increasing in number specifically where hosts are located,[5] though with some limitations such as dependence on relatively high bacterial densities.[3,7,8] We call this auto "dosing" because the phages themselves contribute to establishing the phage dose.[3]

Low inherent toxicity. Since phages consist mostly of nucleic acids and

proteins, they are inherently nontoxic.[3,9,10] However, phages can interact with immune systems, at least potentially resulting in harmful immune responses, though there is little evidence that this actually is a concern during phage treatment.[4,5,11,12] Nonetheless, it can be imperative for certain phage therapy protocols to use highly purified phage preparations[8] to prevent anaphylactic responses to bacterial components, such as the endotoxins that can be found in crude phage lysates.[10] Phages similarly can release bacterial components while killing bacteria in situ, a property associated with lysis that also can result from the application of cell-wall disrupting antibiotics.

Minimal disruption of normal flora. Owing to their host specificity—which can range from an ability to infect only a few strains of a bacterial species to, more rarely, a capacity to infect more than one relatively closely related bacterial genus[13]—phages only minimally impact health-protecting normal flora bacteria.[10,14] By contrast, many chemical antibiotics, which tend to have broader spectrums of activity, are prone to inducing superinfections, such as antibiotic-associated *Clostridium difficile* colitis or *Candida albicans* yeast infections.[5] The historical bias towards developing only broader spectrum antibiotics, however, may be changing.[15]

Narrower potential for inducing resistance. The relatively narrow host range exhibited by most phages[13] limits the number of bacterial types with which selection for specific phage-resistance mechanisms can occur. This contrasts with the substantial fraction of bacteria that can be affected by most chemical antibiotics.[5] In addition, some mutations to resistance negatively impact bacterial fitness or virulence due to loss of pathogenicity-related phage receptors.[7,8]

Lack of cross-resistance with antibiotics. Because phages infect and kill using mechanisms that differ from those of antibiotics, specific antibiotic resistance mechanisms do not translate into mechanisms of phage resistance. Phages consequently can be readily employed to treat antibiotic-resistant infections[5,9-12] such as against multi-drug-resistance *Staphylococcus aureus*.[14,16]

Rapid discovery. Phages against many pathogenic bacteria are easily discovered, often from sewage and other waste materials that contain high bacterial concentrations. Isolation can be more technically demanding, however, if host bacteria themselves are difficult to culture[17] and bacteria may differ in terms of the number of phage types to which they are susceptible.[18] Unlike antibiotics, which can be toxic,[19] phages that display little or no toxicity can be isolated against most target bacteria.

Formulation and application versatility. Phages, like antibiotics, can be versatile in terms of formulation development, such as being combined with certain antibiotics.[9,11] They are also versatile in application form, as liquids, creams, impregnated into solids, etc., in addition to being suitable for most routes of administration.[4,5,9,12,18] Different phages furthermore can be mixed as cocktails to broaden their properties, typically resulting in a collectively greater antibacterial spectrum of activity.[4,9,20]

Biofilm clearance. Biofilms tend to be substantially more resistant to antibiotics than planktonic bacteria. Phages, however, have a demonstrated ability to clear at least some biofilms, perhaps owing to a potential to actively penetrate their way into biofilms by lysing one bacterial layer at a time, or due to the display of biofilm exopolymer-degrading depolymerases.[21]

Additional Advantages of Phage Therapy

The following advantages associated with the use of phages as antibacterials have possible safety-, economic-, or convenience-enhancing virtues but are not essential to phage antibacterial use.

Single-dose potential. Applying phages in only a single dose[7] takes advantage of the phage potential to replicate and thereby achieve 'active' therapy, i.e., significant phage amplification via auto "dosing" that results in greater bacterial kiling.[3] Achieving efficacy following only a single dose, or far less frequent dosing, is an obvious convenience, though in many or most instances a single dosage of phages should not be expected, a priori, to be sufficient to achieve desired efficacy.[7]

Possible transfer of phages between subjects. This is essentially cross-infection of phages from treated subjects or environments to untreated subjects. This could be useful in some agricultural applications.[11,22,23]

Capacity for low-dosage use. The ability of phages to increase in density in situ, given sufficient bacterial densities, could potentially reduce treatment costs by reducing phage doses required to achieve efficacy.[3] Application of phages in low doses may also improve product safety, since phages will only increase in density if they are actively killing bacteria and do not otherwise linger long within the body.[9] Avoiding phage application at higher doses for safety reasons, however, has utility only if phage application at higher doses is *not* safe, but there is little evidence suggesting that higher versus lower phage doses may be associated with increases in side effects, especially when using purified phage preparations.

Single-hit kinetics. Unlike chemical antibiotics, only a single phage is needed to kill a single bacterium.[5] Often fewer "units" of phages therefore are required per treatment, though high multiplicities of phage adsorption to bacteria are still necessary to substantially reduce target bacterial densities.[3]

Low environmental impact. Because they are composed predominantly of nucleic acids and proteins,[3] and possess relatively narrow host ranges,[13] discarded therapeutic phages, unlike broad-spectrum chemical antibiotics,[24] will at worst have an impact on only a small subset of environmental bacteria. Phages not adapted to degradative environmental factors, such as sunlight, desiccation, or temperature extremes, also can be rapidly inactivated.

Phages are not antibiotics. There are a number of non-essential uses of antibiotics that contribute to bacterial evolution of resistance: antibiotic treatment of animal or plant diseases, antibiotic use to increase food-animal growth rates, and over- or improper use of antibiotics to treat human diseases.[4,25,26] In addition, there is concern about antibiotic contamination of foods (e.g., milk) as well as of downstream environments such as from sewage effluent.[24] Since phages do not contribute

to antibiotic resistance, using phages to replace antibiotics could help extend the clinical utility of conventional antibiotics.

Phages are natural products. Public resistance to laboratory-synthesized drugs or genetically modified organisms should not apply to non-engineered phage products as they are natural components of environments.[10]

Relatively low cost. The production of phages predominately involves a combination of host growth and subsequent purification.[27] While the cost of host growth varies depending upon bacterial species, the cost of phage purification appears to be coming down as technology improves.[28] Generally these costs of phage production, per unit,[9] are not out of line with the costs of pharmaceutical production while the costs of discovery (isolation) and characterization can be relatively low.[10]

Potential Disadvantages

Concerns about using phages as antibacterial agents can be distinguished into four categories: (1) phage selection, (2) phage host-range limitations, (3) the "uniqueness" of phages as pharmaceuticals, and (4) unfamiliarity with phages. See references 4 and 5 for additional discussion.

Not all phages make for good therapeutics. Good therapeutic phages should have a high potential to reach and then kill bacteria in combination with a low potential to otherwise negatively modify the environments to which they are applied. These characteristics can be reasonably assured so long as phages are obligately lytic, stable under typical storage conditions and temperatures, subject to appropriate efficacy and safety studies, and, ideally, fully sequenced to confirm the absence of undesirable genes such as toxins.[10,18] Note that a phage that is "obligately lytic" we define as *not temperate and released from infected cells via lysis*, that is, unable to display lysogeny and not released chronically. The use of temperate phages as therapeutics is problematic due to a combination of display of superinfection immunity,[13] which converts phage-sensitive bacteria into insensitive ones, and the encoding of bacterial virulence factors, including bacterial toxins.[8-10,18,27,29]

In addition to avoiding temperate or toxin-carrying phages, the aim of phage characterization is to exclude as therapeutics those phages that display poor killing potential against target bacteria. Such low "virulence" can be due to poor adsorption properties, low potential to evade bacterial defenses, or poor replication characteristics.[3] Also less desirable for therapeutics are those phages that display poor pharmacokinetics, that is, poor absorption, distribution, and survival in situ.[3] Ideally phages should also display a low potential to transfer bacterial genes between bacteria (transduction).[10,18]

Phage characterization additionally can include virion morphology (via electron microscopy), protein profiles, or genotype characterization other than via full-genome sequencing (e.g., PFGE profiles of restriction digested genomes), etc.,[18] though the costs associated with exhaustive phage characterization prior to phage use can be prohibitive. The general aim, therefore, should be to identify those phages that display good primary pharmacodynamics (that is, antibacterial virulence), minimal secondary pharmacodynamics (low potential to do harm to patients), and good pharmacokinetics (an ability to reach target bacteria in situ).[3] Phages that do not adequately meet these criteria should in most circumstances not be employed as therapeutics. Minimally this should entail avoiding temperate phages and, ideally, full genome sequencing should be used to rule out virulence-factor carriage.

The problem of narrow host range. No antimicrobials displaying selective toxicity will affect all possible microbial targets. Typically the narrowness of phage host ranges—a few strains, a few species, or much rarer, a few genera of bacteria[13]—will at a minimum place limitations on presumptive treatment, i.e., treatment courses that begin prior to the identification of the pathogen's susceptibility to antibacterials such as to specific phages. However, as phages can often be employed in combination with other antibacterial agents, including other phages (so-called phage cocktails), the lytic spectrum of phage products can be much broader than the spectrum of activity of individual phage types.[4,9,20] Even broadly acting phage cocktails are normally more selective in their spectrum of activity than typical 'narrow-spectrum' antibiotics, a property that can be viewed as an additional advantage of phage therapy.

Phages are not unique pharmaceuticals… Phages as pharmaceuticals are protein-based, live-biological agents that can potentially interact with the body's immune system, can actively replicate, and can even evolve during manufacture or use, but are far from unique in these regards. For example, many protein-based pharmaceuticals can stimulate immune systems, antibiotics that lyse bacteria will release bacterial toxins in situ, and live-attenuated vaccines both actively replicate and evolve including within the context of *infecting body tissues*. Protein-based drugs, chemical antibiotics, and whole vaccines have previously been approved for use despite these various properties. It therefore stands to reason that phage-based pharmaceuticals should not be disqualified for possessing similar attributes.

…but nonetheless are unusual. The Western medical establishment's unfamiliarity with phages, as antibacterial agents, may be phage therapy's greatest challenge. However, as noted, the various phage oddities as drugs at least are not unique to them. Indeed, a few phage products have now passed regulatory standards, having been classified by the FDA as GRAS (Generally Regarded As Safe), registered by the EPA, or approved for use by the USDA.[9,26] Nevertheless, phages as 'viruses' could be misinterpreted by the general public as being in some manner equivalent to viral pathogens that cause human disease. So far, however, public resistance has not materialized, and it is perhaps fortunate that bacterial viruses are known, instead, as phages.

Conclusion

Phages, as antibacterial agents, have a number of properties that make them compelling alternatives to chemical antibiotics while most or perhaps all concerns associated with phage therapy should be manageable through a combination of proper phage selection, effective formulation, and greater clinician understanding of and familiarity with product application.

Interestingly, a number of the points highlighted in this article were originally considered by d'Hérelle, as described in the translated version of "The phenomenon of the cure in infectious diseases", also published in this issue.[30] Suitable phages, for example, were selected by characterizing their range of antibacterial virulence (narrow or broad), phage stability was confirmed at various temperatures, phage cocktails were developed to presumptively treat acute infections, and new phages were easily isolated against bacterial strains obtained from chronic infections.

In an era where antibiotic-resistant bacterial infections are on the rise, phages provide numerous advantages, along with relatively few disadvantages. In light of science now having a much greater understanding of phage biology along with higher standards for medical investigation than were the case during phage therapy's early, formative years,[1] phage therapy merits a second chance within Western medicine to show its true potential.

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